VegT, ortholog is expressed maternally in these animals as well as zebrafish, mouse and protochordates, suggesting that *VegT* is a maternal factor for endoderm differentiation only in amphibian. The study raises the viewpoint that the lamprey/bichir type holoblastic development would have been ancestral to extant vertebrates and retained in their stem lineage as a preliminary state toward the meroblastic development; amphibian-type holoblastic development would have been acquired secondarily, accompanied by the exploitation of new molecular machinery such as maternal *VegT*.

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Program/Abstract # 244
Changes in localization and expression levels of Shroom2 and spectrins contribute to variation in amphibian egg pigmentation patterns

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One contributing factor in the worldwide decline in amphibian populations is thought to be exposure of eggs to UV light. Enrichment of pigment in the animal hemisphere of eggs laid in the sunlight defends against UV exposure, but less is known about how such mechanisms were modified during evolution to achieve the wide diversity of amphibian egg pigment patterns. Here, we show that ectopic expression of the γ-tubulin regulator, Shroom2, is sufficient to induce co-accumulation of pigment granules, spectrin, and dynactin in *Xenopus* blastomeres. Moreover, Shroom2 and spectrin are enriched and co-localize specifically in the pigmented animal hemisphere of *Xenopus* eggs and blastulae. Moreover, Shroom2 mRNA is expressed maternally at high levels in *Xenopus*. By contrast to *Xenopus*, eggs and blastulae of *Physalaemus pustulosus* have very little surface pigmentation. Rather, we find that pigment is enriched in the perinuclear region of these embryos, where it co-localizes with spectrin. Moreover, maternal Shroom2 mRNA was barely detectable in *Physalaemus*, though zygotic levels were comparable to *Xenopus*. We therefore suggest that a Shroom2/spectrin/dynactin-based mechanism controls pigment localization in amphibian eggs, and that variation in maternal Shroom2 mRNA levels accounts in part for variation in amphibian egg pigment patterns during evolution. Localization and expression levels of Shroom2 and spectrins govern animal hemisphere pigmentation in amphibian eggs.

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Program/Abstract # 245
CXCR4 drives neural crest cells to the sympathetic ganglia

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The proper guidance of neural crest progenitor cells is critical to the development of the vertebrate body plan, including formation of the dorsal root ganglia (DRG) and sympathetic ganglia (SG) of the peripheral nervous system. Trunk neural crest cells (NCCs) are sculpted into discrete migratory streams through rostral somite halves, however it is unclear what molecular mechanisms drive NCCs over long distances to ventral locations within the embryo. Here, we determined a role for chemokine signaling to modulate trunk NCC migration along the ventromedial pathway to the dorsal aorta. Expression analysis by RT-PCR and *in situ* hybridization revealed that a subset of trunk NCCs expressed CXCR4 and the tissue dorsal to the dorsal aorta expressed SDF-1. *In vitro* time-lapse confocal imaging and *in vivo* bead transplantation experiments showed attraction and gathering of NCCs around SDF-1 soaked beads, respectively. Knock down of NCC CXCR4 expression using shRNA revealed disruption of long distance NCC migration and differentiation of sympathetic neurons. Significantly fewer CXCR4-shRNA+ cells reached ventral SG target sites and located to the inner core of SG, a site of neuronal differentiation Thus, CXCR4/SDF-1 signaling plays a vital role in trunk NCC navigation and may be part of a signaling network to sort a common pool of trunk NCCs into the SG and DRG.

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Program/Abstract # 246
Late emerging trunk neural crest cells in the turtle *Trachemys scripta*

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Turtle plastron bones develop by intramembranous ossification from the condensation of cells that stain positively for HNK1, PDGFRα and p75, indicating that these bones are derived, like the facial bones, from neural crest cells. At Greenberg stage 17, comparable to H&H Stage 28 chick embryos and well after the initial wave of neural crest migration, cells that are positive for HNK1 and the early neural crest marker, FoxD3 begin accumulating in the thickened dermis of the carapace and migrating to the developing plastron. We have been able to demonstrate that these cells share the defining attribute of neural crest cells, that of emerging from the neural tube. We injected the lipophilic dye DiI into the lumen of the neural tube of St.17 turtle embryos. Within a day after injection, DiI-positive cells can be seen in the carapacial ridge "staging area" that contains the HNK1-positive cells. Moreover, these cells form migratory streams going away from the dorsum. In addition, we have cultured neural tubes from St.17 embryos, and observed HNK1+ cells migrating away from them. Currently, we are in the process of comparing the molecular and functional properties of these late trunk neural crest cells with those of cranial neural crest cells. These data support our hypothesis that the plastron bones of the turtle are formed by a late emerging population of neural crest cells that collect dorsally in the carapacial dermis and then migrate ventrally.

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Program/Abstract # 247
Preplacodal region marked by Six1 in mice

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The border between neural and non-neural ectoderm gives rise to paired placodes and neural crest. The sensory placodes, transient thickenings of ectodermal epithelium, give rise to cranial sense organs such as the nose and ear, and represent an important source of neural tissue for the ganglia of the cranial nerves and for the lateral lines. Placodes arise from either neural folds themselves or adjacent to the neural crest in the presumptive head. During late gastrulation and early segmentation stages, all placodes develop from contiguous pre- or pan-placodal region (PPR) located around the anterior neural plate.

The PPR is specified by members of two major transcription factors, the Six family and their cofactors, Eya family. Recent evidence suggests that the convergence of multiple activities of signaling molecules, such as FGF, BMP, and WNT is required to induce expression of transcription factors encoded by Six1/2, Six4/5, and Eya genes, emphasizing that they are the earliest markers of PPR fate in *Xenopus*, zebrafish, and chick. In mice, however, little is known about the region of PPR in relation to that of neural crest cells, and the target genes of Six and Eya in the PPR. Here we examined the expression pattern of neural plate, neural crest, PPR, and epidermis marker genes, such as Otx1, Otx2, Msx1, AP2, Snail, Slug, Dlx5, Zic1, and Pax6, together with Six1 as the placodal marker. We found that the expression patterns of several genes are conserved between mouse and *Xenopus*, but others are not. The expression of these genes is also examined by Six1 and Six1/Six4 knockout embryos. Implication of these results will be discussed.

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Program/Abstract # 248
Comparative analysis of SM50 and other genes required for development of the larval skeleton in sea urchins
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Our lab utilizes the sea urchin as a model system to explore the functional consequence of changes in genes and their cis-regulatory elements with regard to protein-binding affinity, patterns of gene expression in the embryo, and/or phenotype. In particular, we are analyzing genes that are required for development of the larval skeleton in the sea urchin. We expect that changes in their transcriptional regulation may be responsible for several differences in the origin and behavior of cells that give rise to the larval skeleton and its morphology among different species of sea urchins. During the past few years, our lab has been investigating the functional significance of variation in the cis-regulatory region of *SM50* within the purple urchin, *Strongylocentrotus purpuratus*. In this study, we have begun to investigate the functional significance of similarities and differences in the cis-regulatory region of *SM50* among several closely related species. In particular, we have identified a ~100 bp region that is well conserved between *S. purpuratus* and the white urchin, *Lytechinus variegatus*. We are using site-directed mutagenesis to explore the role of this evolutionarily conserved region and putative cis-regulatory elements that occur within it. In addition, we have begun to extent our work to other genes as well as more distantly related species such as the pencil urchin, *Eucidaris tribuloides*. We have isolated a few genes including Erg from *E. tribuloides*. We expect that this work will eventually provide us with insight into the molecular basis of diversity.

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Program/Abstract # 249
Mesenchymal regulation of vascular invasion and matrix remodeling during intramembranous ossification
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With the goal of devising new treatments for skeletal disease and injury, there is much need to discover mechanisms through which mesenchyme makes bone. Many studies have pinpointed molecular factors that induce the osteogenic differentiation of mesenchyme in vitro, but given that a clinical objective is to engineer mesenchyme for applications in vivo, more work is required to identify critical interactions between osteogenic mesenchyme and surrounding tissues. We investigate the extent to which mesenchyme regulates vascular invasion and matrix remodeling. In the developing jaws and face, all bone comes from neural crest mesenchyme (NCM) whereas angioblasts and osteoclasts arise from mesoderm. We take advantage of these distinct embryonic origins and transplant faster developing quail NCM into slower developing duck embryos. Previously, using this quail-duck chimeric system, we have shown that NCM makes bone by executing autonomous molecular and histogenic programs. Here, we assess the effects of NCM on host-derived blood vessels and osteoclasts using gene expression, histology, and whole-mount staining analyses. We find that quail donor-NCM induces premature vascular invasion by the duck host during ossification but not at earlier stages. NCM also influences osteoclast activity in a manner that has direct implications for species-specific differences in the size of skeletal elements. We conclude that NCM plays an important role in synchronizing the timing of vascular invasion and matrix remodeling.

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Program/Abstract # 250
Mesenchymal regulation of mineralization and bone mineral density in the jaw skeleton
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Proper bone mineral density (BMD) is crucial for skeletal function. Loss of BMD can lead to osteoporosis and fractures. From an evolutionary perspective, BMD helps couple morphology to the environment. For example, in aquatic mammals a higher BMD enables negative buoyancy during diving. BMD is governed by systemic and local factors throughout the life of an organism. On a cellular level, BMD is affected by osteoblasts, which secrete bone matrix that then mineralizes. During craniofacial development, osteoblasts are derived from neural crest mesenchyme (NCM). We use an avian chimeric system that exploits differences in morphology and maturation rates between quail and duck to investigate the role of NCM in establishing BMD. We unilaterally transplant quail NCM into duck, which maintains the host side as an internal control, and we analyze mineralization at the molecular and histological levels. We assess mineral deposition rates in quail, duck, and chimeric embryos by injecting fluorescent labels and by whole-mount staining. We also measure BMD using DEXA and MicroCT. We find that duck have a slower rate and longer period of mineralization correlated with higher BMD, which is significant since duck use their bills for filter feeding and for diving. In chimeras, quail donor NCM alters the rate of mineral deposition and BMD. Thus, species-specific differences in BMD have likely evolved through NCM-mediated changes in molecular programs underlying mineralization.

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Program/Abstract # 251
A gain of function mutation causing skeletal overgrowth in the *rapunzel* mutant
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